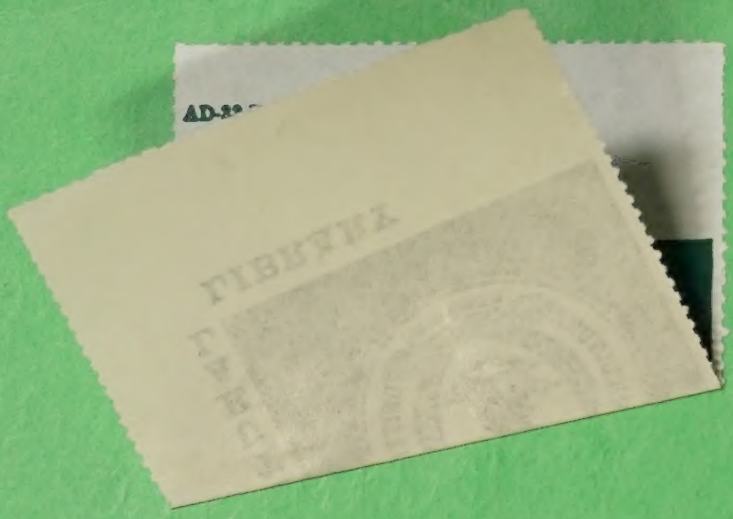
Beltsville Symposium VII

Genetic Engineering: Applications to Agriculture

May 16-19, 1982

ABSTRACTS



ABSTRACT SESSION
MAY 17, 1982
Conservatory
1:30 - 4:30 P.M.

1. Authors of odd-numbered abstracts will be attending their posters at 1:30-2:30 p.m.
2. Authors of even-numbered abstracts will be attending their posters at 2:30 - 3:30 p.m.
3. Abstracts 1-46 will be presented as posters, 48-56 will not.
4. Abstract 47 will be presented in the hall of Building 003 as an on-line computer demonstration.
5. Abstracts 57-71 will be presented as Symposium overview lectures.

1

CHROMOSOME MOBILIZATION PLASMIDS IN RHIZOBIUM JAPONICUM

Atherly, Alan G.* and James O. Berry. Genetics Dept., Iowa State Univ., Ames, IA 50011 USA

Several P-group plasmids (R68.45, R772, RP4, RP1) were introduced into R. japonicum strains USDA 31, 110 and strains 61A76 and AA102. Two procedures were used for plasmid introduction: conjugation from an E. coli donor or by transformation of spheroplasts. Considerable data indicate that the plasmids are present in the new host; however, in strains 110,31 and 61A76 agarose gel electrophoresis of DNA from cell extracts did not reveal the presence of P-group plasmids. On the other hand, the large endogenous plasmids were clearly visable. When P-group plasmids were introduced into R. japonicum by spheroplast transformation, the endogenous plasmid increased in molecular weight corresponding to the size of the introduced plasmid (40 mdal). In contrast, when P-1 group plasmids were introduced into R. japonicum by conjugation, no increase in endogenous plasmid molecular weight was seen.

P-group plasmid transfer between R. japonicum strains and into E. coli or Pseudomonas aeruginosa strains occurred at a high frequency (1×10^{-3} to 1×10^{-5}) but we have not yet been able to obtain gene transfer between R japonicum strains.

2

CYTOPLAST FORMATION AND ENRICHMENT FROM MESOPHYLL TISSUES OF NICOTIANA SPP.

Archer, E. Kathleen*; Craig R. Landgren & Howard T. Bonnett, Biology Dept., University of Oregon, Eugene, OR 97403 USA.

Cytoplasts are cytoplasmic derivatives lacking a nucleus, but containing plastids and mitochondria. Protoplast preparations from leaves of Nicotiana (deb)tbc were found to contain 10-15% cytoplasts. Suspension culture cells of N. (deb)tbc, which are more isodiametric than elongate, produced no cytoplasts, while suspension cultures of N. glutinosa, which contain many elongated cells, did produce cytoplasts. In N. glauca, isolates from young leaves produced fewer cytoplasts than mature leaves which have more elongated cells. We used onion epidermis as a model system to observe cytoplast formation during plasmolysis, and our observations suggest that cytoplasts may be obtained from plant tissues provided that elongated cells are present. We have used density gradient centrifugation to enrich preparations for cytoplasts by 3-5 fold. Such preparations may be useful in the production of plant cybrids through cytoplast-protoplast fusions.

3

A Simple Method For Cloning And Selection of Specific DNA Sequences in pBR322: Molecular Cloning of Adenovirus Type2 DNA

S. Bhaduri, Eastern Regional Research Center, Agricultural Research Service, USDA, Philadelphia, PA 19118

A technique has been developed for cloning fragments of adenovirus type2 DNA (Ad2), using pBR322 as vector following digestion by a pair of restriction endonucleases. pBR322 contains unique EcoR1, BamH1 and HindIII restriction sites. Some restriction sites including BamH1 and HindIII are located within the tetracycline resistance gene; insertion of a DNA fragment at these locations causes loss of tetracycline resistance (insertional inactivation). Restriction endonuclease fragment EcoR1B and BamC of Ad2 DNA were cleaved with BamH1 and EcoR1 respectively. The resulting fragments, containing both an EcoR1 and a BamH1 end, were inserted into pBR322 which had been doubly digested with BamH1 and EcoR1. Similarly, EcoR1F of Ad2 DNA was cleaved with HindIII and subsequently inserted into HindIII and EcoR1 digested pBR322. Hybrid plasmids constructed by this method still retain ampicillin resistance in pBR322, allowing selection of transformants. Subsequent screening for tetracycline sensitivity facilitates identification of strains harboring chimeric plasmids. The presence of Ad2 DNA sequences in the hybrid plasmids was further confirmed by 1) colony hydrization II) hybridization to late AD2 RNA and III) restriction endonuclease analysis.

4

ASPECTS OF EMBRYOGENESIS IN MEDICAGO SATIVA L. TISSUE CULTURES

Atanassov, Atanas I.*[a] and Daniel C. W. Brown, Agriculture Canada, Genetic Engineering Section, Ottawa Research Station, Ottawa, Canada K1A 0C6. [a]Bulgarian Academy of Science, Institute of Genetics, Sofia 1113, Bulgaria.

In the first phase of a program exploring the use of tissue culture technology for forage crop improvement, a number of alfalfa cultivars in use in Canada were surveyed for in vitro regeneration capacity. Various B_5 based media (Gamborg et al., Exp. Cell Res. 50:151-158, 1968) were found to induce and support callus growth on explants of leaf cotyledon and hypocotyl tissue. One of the most successful media was B_5 modified to contain 30,000 mg/L sucrose, 2,100 mg/L $CaCl_2.2\ H_2O$, 800 mg/L glutamine, 100 mg/L serine, 10 mg/L glutathione, 1.0 mg/L adenine, 1.0 mg/L 2,4-D, 0.2 mg/L kinetin. Evidence of embryogenesis in cell suspension cultures has been found in many of the cultivars tested to date. These include Answer, Armor, Banner, Citation, Iroquois, Multileaf, Peak, Pioneer 520, Rambler, Saranac and Thor as well as the Regen S strain of Saranac obtained from E. T. Bingham, University of Wisconsin. Work is continuing on the screening of material derived from protoplasts of leaf, callus and cells grown in suspension cultures.

5

NON-RANDOM LOSS OF REPETITIVE DNA SEQUENCES IN SOYBEAN COTYLEDONS DURING SENESCENCE

Chang, D-Y.* and J. P. Miksche, Botany Dept., NCSU, Raleigh, NC 27650 USA

Fragments of repetitive DNA from soybean cotyledons were isolated and cloned in E. coli cells. These cloned DNA sequences were used as probes to detect DNA changes in senescing cotyledons. The DNA sequences which were lost in senescing cotyledons were selected by the colony hybridization. Southern blot hybridization between selected cloned DNAs on filters and total DNA from different cotyledon senescing stages showed that the disappearance of DNA sequences was not a random event. The hybridization between total DNA and ^{32}P- labeled cloned DNA was used to estimate the copy number and percentage of these sequences in the soybean genome. Time course studies of copy number changes in the soybean genome indicated that profiles of decreasing DNA in senescing cotyledons were different among the measured sequences. The results of these studies suggested that the repetitive DNA sequences were degraded selectively in the genome of senescing cotyledons and the susceptibility of DNA to the degradation may be correlated to the organization of DNA sequences.

6

SOMATIC HYBRIDIZATION IN NICOTIANA. Bonnett, H.* & K. Glimelius. Dept. Biology, U. of Oregon, Eugene, OR. 97403 & Institute of Physiological Botany, U. of Uppsala, Uppsala, SWE.

Hybrids have been regenerated from fused protoplasts isolated from cell cultures of a male fertile cultivar of Nicotiana tabacum deficient in nitrate reductase (Müller and Grafe, 1978, Mol. Gen. Genet. 161:67) and from three different male sterile cultivars. Mature plants were analyzed for the following organellar genetic traits: ribulose-bisphosphate carboxylase (RuBP-case), tentoxin sensitivity, and cytoplasmic male sterility. Most hybrids from all three of the crosses showed restoration of stamen production, thereby displaying the fertility trait from the mitochondria of the nitrate reductase line. Analysis of the two traits coded by chloroplast DNA, RuBP-case and tentoxin sensitivity, indicated that segregation of chloroplasts occurred in nearly all cases by the time of plant regeneration. No new combinations of these two traits, which would indicate recombination of chloroplast material, were found. The frequency with which each chloroplast type occurred among the hybrids differed according to which male sterile cultivar was used. Such differences indicate that the origin of the chloroplasts determines the frequency (i.e., competitive ability) of occurrence in hybrid plants. These differences will be compared to the structural similarities between Nicotiana chloroplast DNAs as determined by Kung, Zhu, and Shen, 1981, Theor. Appl. Genet. 61:73.

7

TRANSFER RNA GENE MAPPING STUDIES ON THE ORGANELLAR DNA FROM VARIOUS PHOTOSYNTHETIC ORGANISMS

Crouse, Edwin J.*, Mfika Mubumbila, Marcel Kuntz, Mario Keller, Karl H.J. Gordon, Gerard Burkard, Andre Steinmetz & Jacques H. Weil, IBMC, 15 rue Descartes, 67084 Strasbourg, France
Richard Selden, Lee McIntosh, Lawrence Bogorad, Harvard University, 16 Divinity Avenue, Cambridge, MA 02138 USA
Wolfgang Löffelhardt & Herrmann Mucke, Institut für Biochimie, Währingerstrasse 38, 1090 Wien, Austria
Hans J. Bohnert, EMBL, Postfach 10.2209, 6900 Heidelberg, West Germany

Transfer RNAs have been isolated from the chloroplasts of a dicotyledon (Phaseolus vulgaris), a monocotyledon (Zea mays), a green alga (Euglena gracilis), and from the cyanelles of Cyanophora paradoxa by two-dimensional gel electrophoresis, identified by aminoacylation using E.coli aminoacyl-tRNA synthetases and ^{3}H-amino acids, labelled in vitro with ^{32}P and used for hybridization with the corresponding organellar DNA restriction fragments. A comparison of the tRNA gene maps of these four genomes will be presented.

8

IN VITRO REGENERATION OF *NICOTIANA ALATA*

Bravo*, Janis E. and David A. Evans, DNA Plant Technology Corporation, Cinnaminson, New Jersey 08077

Plants were regenerated from leaf explants of *Nicotiana alata* cultured on MS agar media containing various concentrations of 6-benzylaminopurine (6BA). 25% of the plants regenerated on 5µM and 10µM 6BA were tetraploid. These were crossed with diploid plants to produce stable triploids. Variability with respect to time to flowering, flower and leaf morphology, and pollen viability was observed among regenerates. Plants were also regenerated from petal explants of *N. alata* cultured on MS agar media containing 5µM 6BA.

9

PHYTOCHROME REGULATION OF SPECIFIC CYTOPLASMIC AND CHLOROPLAST RNAs IN PEA AND MUNG BEAN SEEDLINGS

Everett, Marylee, Richard A. Jorgensen, Jeffrey D. Palmer & William F. Thompson*, Dept. of Plant Biology, Carnegie Inst. of Washington, Stanford, CA 94305

We have examined phytochrome effects on the abundance of transcripts from several nuclear and chloroplast genes in dark-grown pea buds and mung bean primary leaves. The genes studied include those coding for nuclear and chloroplast ribosomal RNA, both subunits of RuBP carboxylase, the chlorophyll *a/b* binding protein, and a 32,000 dalton photosystem II polypeptide as well as a number of others whose function is presently unknown but which encode RNAs whose abundance is regulated by light. Cytoplasmic mRNAs are represented by clones selected from a cDNA library in pBR322 and chloroplast mRNAs by genomic fragments. Classical red/far-red reversible phytochrome responses are observed, although the major effect of red light is to accelerate a much larger subsequent response to high intensity white light. Clear differences exist in the pattern of induction for at least two groups of cytoplasmic and chloroplast mRNAs, and between peas and mung beans.

10

MARKERS OF THE PROCESS OF IN VITRO SHOOT PRODUCTION

Christianson, Michael L.*& Debra A. Warnick, Department of Molecular Biology, Zoecon Corporation, Palo Alto, CA 94304 USA

Leaf explants of Convolvulus arvensis produce shoots when placed on a simple medium: Murashige and Skoog salts, sucrose, vitamins, 0.05 mg/l IAA and 7.0 mg/l 2ip. We can divide the time between placement of the explants on the medium and the appearance of elongating shoots into a number of operationally defined steps: transfer of explants after various times in culture shows the effect of many inhibitors or promotors of regeneration to be time-specific. In some cases, mechanisms shown or suggested for the inhibitor molecule in other systems serve as clues to the nature of gene action during shoot formation. Coupled with histological examination and direct assays for metabolites, notably starch and amino acids, these operationally defined steps become a detailed picture of the process of regeneration. Although there are differences between individual plants in the absolute time in culture at which a particular step occurs, the developmental sequence of the steps is conserved. We believe the identification of the component steps of the process of in vitro shoot formation will facilitate the elaboration of media sequences to give plant regeneration from species for which regeneration has not yet been achieved.

11

cDNA CLONING OF THE GENES FOR ENVELOPE GLYCOPROTEINS FROM NEWCASTLE DISEASE VIRUS

Fox, Lawrence M.*, Department of Molecular Genetics, Hoffmann-La Roche Inc., Nutley, NJ 07110 USA

I have initiated a project to determine the genetic basis of strain specific differences in virulence of Newcastle disease viruses (NDV), and to use recombinant DNA technology to produce pure viral envelope proteins with potential value as vaccines. NDV RNA (strain B1) was converted into cDNA and cloned into pBR322. Based on the available amino acid sequence of the F1 glycoprotein, a synthetic oligonucleotide will be used to identify clones containing the Fo glycoprotein gene. This gene will be sequenced and engineered for expression in E. coli, yeast and mammalian cells. The DNA sequence of the Fo gene derived from strain B1 will be compared to a similarly obtained clone from strain California to determine the genetic basis of the strain differences in proteolytic activation of the F-glycoproteins. Ultimately, the proteins expressed in various hosts will be compared in their efficacy as vaccines.

12

POTENTIAL OF ASEPTIC CULTURE TECHNIQUES FOR BANANA IMPROVEMENT

Cronauer, Sandra S.* & A.D. Krikorian, Biochemistry Dept., State Univ. of N.Y. at Stony Brook 11794 USA

Bananas and plantains are among the most sterile of cultivated crops and opportunities for genetic improvement are quite limited. We are assessing the potential value of tissue, cell and protoplast culture techniques for expanding the available options. Shoot tip cultures can provide axenic material with which to work. Apices isolated from mature plants or suckers may be cultured on any of several media such as the basal salt medium of Linsmaier and Skoog supplemented with BAP (5 mg/l), inositol (1000 mg/l), and sucrose (4%). A single apex usually produces a single shoot or plant but formation of multiple shoots can be stimulated by longitudinally splitting the shoot through the apex. Roots can be induced to form on individual shoots within a few days by exposure to activated charcoal (0.25%). While these rapidly multiplied small plantlets may be used for mutation breeding by exposure to chemical mutagens and irradiation, we have used these clones as a source of aseptic cells and protoplasts. A report will be provided on the progress made thus far.

13

Virus-like characteristics of the agent associated with Cytoplasmic Male Sterility in the Vicia faba "447" line.
Grill, L. K.*, T. H. Turpen, and S. J. Garger. Department of Molecular Biology, Zoecon Corporation, 975 California Avenue, Palo Alto, CA 94304

It has now been well documented that cytoplasmic spherical bodies containing double-stranded RNA are associated with, and most-likely the cause of, cytoplasmic male sterility in the "447" cytoplasmic male sterile (CMS) line of Vicia faba (1,2,3). We have shown that the CMS trait, as well as the CMS-associated dsRNA, can be transferred from a male sterile donor plant to a fertile plant (2). However, many characteristics of the CMS-associated virus-like entity do not allow it to fit into any existing plant virus class. The only disease symptom of the putative agent is the malformation of the anthers and pollen, with otherwise similar normal growth characteristics of the plants. Our data suggest that this infectious virus-like agent is not encapsidated as are other plant viruses. This characteristic, as well as others that have been elucidated, allow us to suggest setting up a new class description for such entities.

(1) Edwardson, J. R., D. A. Bond and R. G. Christie, Genetics 82:443 (1976).
(2) Grill, L. K. and S. J. Garger, Proc. Natl. Acad. Sci. USA 78:7043 (1981).
(3) Scalla, R., G. Duc, J. Rigaud, A. Lefebvre and R. Meignoz, Pl. Sci. Letters 22:269 (1981).

16

CALLUS-INDUCED CHROMOSOMAL MIXOPLOIDY IN REGENERANTS OF INTERGENERIC HYBRIDS
George Fedak, Ottawa Research Station, Agriculture Canada, Ottawa, Ontario K1A 0C6 Canada.

Intergeneric hybrids between Triticum crassum (Boiss.) Aitch. and Hensl. ($2n=6x=42$) and Hordeum vulgare L. ($2n=2x=14$) cv. Bomi had the expected chromosome number of 28, were vegetatively vigorous but sterile. Callus was induced on minute (1-3 cm.) immature inflorescence cultured on Kaos medium with 5 mg./L. of 2,4-D and plantlets regenerated on auxin free medium. First cycle regenerants were mixoploids with chromosome numbers of 28-98 between cells within a plant with dicentrics and fragments in most cells. By the fourth subculture the range in chromosome number had decreased to 35-72 and plants were fertile to backcrossing. Average meiotic configurations at metaphase I of fourth cycle regenerants was $16.0^{I} + 14.9^{II} + 1.5^{III} + .3^{IV}$; the univalents were excluded from nuclei forming micronuclei at an average frequency of 25.03 per quartet. Chromosome pairing in T. crassum is under genetic control and the observed abnormal meiotic behaviour was probably caused by the elimination of chromosomes with meiotic pairing control properties. Nevertheless, the propogation of intergeneric hybrids through callus induction followed by plant regeneration offers the potential for chromosome doubling and fertility restoration.

14

MONOCLONAL ANTIBODY PRODUCTION AGAINST AVIAN COCCIDIA
Danforth, H. D.* and P. C. Augustine, USDA, ARS, Animal Parasitology Institute, Beltsville, MD 20705

Hybridoma cell lines, that secrete antibodies directed against coccidial parasites that infect chickens, turkeys, and quail, have been produced by fusion of mouse myeloma cells with spleen cells from sporozoite-immunized mice. The antibodies demonstrated eight different binding patterns on or in air-dried sporozoites of all species studied as determined by the indirect immunofluorescent antibody test. The patterns varied from a general internal fluorescence, similar to that seen in sporozoites exposed to hyperimmune chicken serum, to localized fluorescence on the tip, pellicle, and refractile body of the parasites. A number of cloned antibody-secreting cell lines have been established for the species of coccidia infecting chickens. Some of these monoclonal antibodies are species- and developmental stage- specific while some cross-react with other species of coccidia and developmental stages. Some monoclonal antibodies inhibit sporozoite penetration into host cells, or block the in vitro development of the parasite once it enters the cell. Development of these monoclonal antibodies facilitates diagnosis of the species of coccidia found in litter samples from field operations. Efforts are currently underway to develop screening assays using hybridoma antibodies to determine flock immunity to coccidiosis, and to characterize and purify coccidial antigen for use in possible vaccine development.

17

PURIFICATION AND CHARACTERIZATION OF A CHLOROPLAST DNA POLYMERASE
R.L. McKown* and K.K. Tewari, Department of Molecular Biology and Biochemistry, University of California, Irvine, Irvine, California 92717, USA.

A DNA polymerase has been purified over 3,000 fold from chloroplasts of pea plants by chromatography on DEAE cellulose, phosphocellulose, DNA agarose, and sedimentation in a glycerol gradient. Sodium dodecyl sulfate gel analyses of the final fraction produced a single discernible protein band with a molecular weight of 90,000 daltons. Gel filtration on sephacryl S-200 and glycerol gradient sedimentation under non-denaturing conditions demonstrate that the chloroplast DNA polymerase consists of a single polypeptide with a native molecular weight of approximately 87,000 daltons. The purified polymerase lacks any endonuclease or exonuclease associated activity. The enzyme activity is inhibited by N-ethylmaleimide (74% at 1.0 mM), ethidium bromide (90% at 0.023 mM), and is resistant to aphidicolin. The purified enzyme is totally dependent on the presence of added DNA, has an absolute requirement for Mg^{++} (12 mM optimal), is stimulated by K^{+} (120 mM optimal), and requires all four deoxynucleoside triphosphates for maximum activity. Native DNA which has been degraded to a limited extent with DNase I is the most efficient primer.

15

LASSO STRUCTURE OF SMALL DNA MOLECULES FOUND IN MITOCHONDRIA FROM PENNISETUM AMERICANUM.
Kim, Byung Dong*, Richard R. Ramsay & Kiljae Lee, Florida State University, Tallahassee, FL 32306.

Low molecular weight DNA in mitochondria from corn and other higher plants has been reported to be of either linear, open circle, or supercoil conformation. During an electron microscopic survey with the aim of identifying size and conformation of mitochondrial DNA we observed previously unidentified structures of small DNA molecules from pearl millet. These included double stranded "lasso"-like structures and linear molecules with covalently bound proteins attached to one end. Linear molecules fell into multimeric size groups (1x,2x,4x), the smallest molecule 0.8 μm. These relationships suggest the rolling hairpin model of DNA replication. The loops of the "lasso" structures ranged from very small to almost the size of the curcular DNA. Even though the molecular mechanism is a subject of further study, this "lasso" structure is an apparent intermediate for circularization of the linear DNA molecule. It may play a significant function in DNA replication and/or transcription.

18

GENERATION OF DAYLILY PLANTLETS FROM CELLS GROWN IN SUSPENSION
Fitter, Mindy S.* & A.D. Krikorian, Biochemistry Dept., State Univ. of N.Y. at Stony Brook 11794 USA

The potential of protoplasts for genetic modification of daylily is no doubt enormous. However, if such genetic manipulations are to have any real value, the generation of viable plantlets (in great numbers) is essential. For several years, this laboratory has been studying the enzymatic isolation, culture and regeneration of daylily protoplasts. Protoplasts are routinely isolated from morphogenetically competent cell suspensions (7-12 days old) using an enzyme mixture (1% Cellulysin, 0.5% Macerase, 1% Rhozyme) including 4.5 mM calcium chloride, 0.3 M each of sorbitol and mannitol in the basal medium of Murashige and Skoog at pH 5.7 with 3 mM MES. The protoplasts are cultured in liquid medium at 22 C. Cells with regenerated walls can be multiplied in quantity in a suspension culture and organized plantlets have been produced from these cultures. These plants appear to be phenotypically identical to plants derived clonally from cell suspension. One plant has bloomed to date. A karyological study of these plants is in progress.

19

NATURAL GENETIC ENGINEERING STUDIES IN THE CONTROL OF DUTCH ELM DISEASE.

H.M.Mazzone, Forest Insect and Disease Laboratory, U.S. Dept. of Agriculture-Forest Service, Hamden, CT 06514 USA

Dutch elm disease (DED) is one of the most devastating tree diseases in history. Its damage to elms has gone virtually unchecked since the introduction in the United States some sixty years ago of the causal fungus. One approach to controlling DED is through genetic engineering studies involving the beetle vector. The beetle could be used as the sole carbon source in growth media for bacteria isolated from its natural microbial flora and for bacteria isolated from diseased beetles. As the sole carbon source for this mixed bacterial culture, the beetle material could be used in small amounts at first. The surviving microorganisms could then be exposed to ever increasing amounts of beetle material in growth media until a sustainable culture was obtained. It is hope that genes of the plasmids of the pathogenic bacteria would pass on to the bacteria which are a part of the natural microbial flora of the beetle.

20

SELECTION OF HETEROKARYONS BASED ON FLUORESCENCE

Galbraith,*David W. & Kristi R. Harkins School of Life Sciences, University of Nebraska-Lincoln, Lincoln, NE 68588 USA.

We have developed procedures for the selection of heterokaryons formed by protoplast fusion based on the detection of (a) exogenous fluorescent probes, derivatives of fluorescein and rhodamine, added to protoplasts derived from mesophyll or tissue (suspension) cultured cells of Nicotiana spp. or (b) endogenous chlorophyll fluorescence. The labeling procedures are non-toxic and do not prevent morphogenesis of calluses derived from the protoplasts. We have been directly selecting the heterokaryons by use of a Coulter Electronics EPICS V Cell Sorter. Under the desired conditions, the process of heterokaryon sorting does not affect viability. We demonstrate that the cell sorter can accurately discriminate between parental and fused protoplasts. The future application of these techniques will be discussed.

21

LEAF SKELETON HYBRIDIZATION ASSESSES CAULIFLOWER MOSAIC VIRUS HOST RANGE.

U. Melcher*, C. Brannan, C. O. Gardner, Jr., and R. C. Essenberg. Department of Biochemistry, Oklahoma State University, Stillwater, OK 74078

Local lesions induced by inoculation of leaves of turnips with cauliflower mosaic virus (CaMV) or its DNA were detected by molecular hybridization with radioactive CaMV DNA. Leaves 10 to 14 days after inoculation were extracted with 2-methoxyethanol, digested with proteinase K, treated with alkali and neutralized. The resulting leaf skeletons were incubated with nick translated ^{32}P CaMV DNA at 68°C for 24 h. After washing and autoradiography circular areas of blackening were detected on the film at positions that corresponded to local lesions identified by staining for starch. When radioactive SV40 or pBR322 DNA was used as a probe no lesions were detected. Using this method, we found that CaMV DNA is capable of forming local lesions on leaves of plant species previously not thought to be hosts for this virus. These species include cotton, soybean, peanut, cucumber, lettuce, tomato and spinach. In no case were visible symptoms of infection detected. Preliminary experiments using leaf skeleton hybridization indicate that CaMV DNA can act as a vehicle for replication of passenger DNA of reasonable size. (Supported by the Oklahoma Agricultural Experiment Station and a Competitive Research Grant from USDA.)

22

IDENTIFICATION OF FUNCTIONAL ANTIGENS OF TRICHINELLA SPIRALIS USING MONOCLONAL ANTIBODIES.

H.R. Gamble$_1$* C.E. Graham$_1$, K.W. Hunter$_2$ and K.D. Murrell$_1$, $_1$Animal Parasitology Institute, NRPDL, Beltsville, MD 20705 and $_2$Dept. of Pediatrics USUHS, Bethesda, MD 20814.

Antibody-secreting hybridomas were obtained by fusing myeloma cells (P3X63 Ag8.653) with spleen cells from Balb/cJ mice infected orally with Trichinella spiralis muscle larvae, or immunized intraperitoneally with an extract of T. spiralis in alum or intramuscularly with an extract of T. spiralis in adjuvant. Fused cells identified as secretors by an anti-kappa chain enzyme immunosorbent assay were selected and screened for isotype. Antigen specificity was determined using a three antibody enzyme immunosorbent assay performed on nitrocellulose blots of electrophoresed T. spiralis protein extracts. The potential use of antigens identified by these methods as diagnostic or vaccinating reagents for swine trichinosis will be discussed.

23

TRANSFORMATION OF YEAST USING LIPOSOME-ENCAPSULATED DNA

Mettler, Irvin J.* & P. A. Okubara, Stauffer Chemical Co., 1200 So. 47th St., Richmond, CA 94804 USA

Liposome encapsulation of DNA could offer an ideal system to deliver DNA to isolated plant protoplasts for genetic engineering studies. We have used isolated yeast spheroplasts as a model system for examining transformation parameters using liposome-encapsulated DNA. Yeast spheroplasts (*Sac. cerevisiae*, strain DBY-746, which requires leucine for growth) were obtained by incubation with cell wall degrading enzymes. Spheroplasts were incubated with YEP-13 plasmid (containing a gene for leucine synthesis) and scored for transformation by the formation of colonies on selective media. The standard method for yeast transformation involves the addition of 10mM $CaCl_2$ with the plasmid DNA, 20 min. incubation, and then the addition of 40% PEG-4000 to induce uptake of the DNA-Ca precipitate by the spheroplasts. Transformation by liposome-encapsulated DNA would require fusion of the liposome with the plasma membrane and release of the DNA to the cytoplasm. Several parameters are being tested in order to maximize the efficiency of liposome-mediated transformation. Control plates, containing leucine, have shown no obvious reductions in cell viability due to the liposome treatments. The use of yeast spheroplasts may provide a useful model system for testing the capability of liposomes to deliver genetic information to plant cells.

24

HYBRIDOMAS PRODUCING MONOCLONAL ANTIBODIES AGAINST PRUNUS NECROTIC RINGSPOT (NRSV) AND APPLE MOSAIC (ApMV) VIRUSES.

HALK, E. L.*, H. T. HSU, J. AEBIG, AND K. CHANG. American Type Culture Collection, 12301 Parklawn Dr., Rockville, MD 20852

Hybridomas secreting monoclonal antibodies were produced by fusing spleen cells from BALB/c mice immunized with a mixture of NRSV and ApMV to cells of mouse myeloma lines NS1/1 or P3X63Ag8.653. Five hybridoma clones secreted antibodies against NRSV, three secreted antibodies against ApMV and one clone secreted antibody that reacted with both NRSV and ApMV. Antibody titers of cell culture media, measured by indirect ELISA, ranged from 1/25 to 1/4,000 whereas antibody titer of mouse ascites fluid ranged from 1/62,500 to 1/39,000,000. Antibodies from clone 70-C9 had ELISA titers >1/310,000 against NRSV-G, Danish plum line pattern virus and ApMV-P and an ELISA titer of 1/2,500 against two ApMV isolates from rose and one isolate from plum. Antibodies from clone 63-F10 reacted only with NRSV-G, and high titered antibodies from clones 74-F11 and 63-E10 reacted with all four ApMV isolates.

25
COMPARATIVE PROPERTIES OF VARIOUS HYBRIDIZATION PROBES IN THE DETECTION OF PLANT PATHOGENS
Odell, Joan T., The Rockefeller University, New York, NY 10021 USA

Detection of plant pathogens at the nucleic acid level has become an increasingly important assay. Techniques involving hybridization to plant nucleic acids attached to DBM- or nitrocellulose paper with various probes need to be rigorously characterized with respect to their comparative sensitivities. Probes include (a) isolated nucleic acids of the pathogen, either labeled in vivo with ^{32}P or following isolation, with ^{125}I; (b) complementary DNA synthesized using pathogen nucleic acid template, suitable primer, and reverse transcriptase and labeled with alpha ^{32}P dNTP's of high specific radioactivity; (c) cloned DNA's nick-translated with DNA polymerase I in the presence of alpha ^{32}P dNTP's; (d) DNA's as in (b) and (c) synthesized in the presence of newly-available biotinated dNTP's. With probes (a) - (c), autoradiography following hybridization is used to detect relevant nucleic acids, while with (d) an antibody detection system is employed. Comparisons of these approaches for detection of sub-nanogram amounts of plant pathogen RNA and DNA using the PSTV system will be discussed, and their relative sensitivities firmly established.

26
PROGENY ANALYSES OF ANDROGENETIC LINES AND INDUCED MUTANTS FROM HAPLOID CULTURES OF BRASSICA NAPUS (RAPE-SEED)
Franz J. Hoffmann, Dept. Developm. & Cell Biol., University of California Irvine, Irvine, Cal. 92717, USA

Progeny analysis of androgenetic plants from inbred rape-seed shows that selective growth of microspores can occur in cultured anthers. The property of privileged growth in culture seems to be linked to such characters as flowering time and seed glucosinolate content which can be analyzed in regenerated plants. This type of selection and the fact that more variability is visible in regenerants from different microspores than in the progeny of the highly inbred anther donor line, demonstrates the higher degree of homozygosity in the doubled amphihaploids. Furthermore, it is shown that haploid genomes of rape may be mutable. Thus it is possible to obtain several different homozygous lines from a single microspore. A system of haploid embryoids arising from single cells of the primary microspore regenerant has also been used to produce experimentally induced mutants. It is demonstrated that recessive mutations can be obtained in a homozygous state in doubled amphihaploid regenerants from mutagenized single cells. The rate of induction of embryoids as well as the applicability of the method is steadily beeing improved in new experiments with several diverse genotypes. Analysis of these plants is in progress at the Max-Planck-Institut für Zellbiologie at Ladenburg, FRG. Hoffmann F, Thomas E, Wenzel G (1982) Theor Appl Genet 61: in press

27
PHOTODEPENDENT GENES IN PEA CHLOROPLAST DNA
Oishi, Karen K.* and K.K. Tewari, Molecular Biology and Biochemistry Dept., University of California, Irvine, Irvine, CA 92717, USA.

Two photodependent genes have been identified and localized on the restriction endonuclease map of pea chloroplast DNA. Total RNA was isolated from dark grown and light grown plants, radioactively end-labeled in vitro by polynucleotide kinase and gamma-^{32}P labeled ATP and hybridized to Southern transfer of pea ctDNA digested with EcoRI. There were two distinct EcoRI DNA fragments of 5.5 Kilo base pair (Kbp) and 4.8 Kbp that did not hybridize with RNA from dark grown plants. These EcoRI DNA fragments showed strong hybridization with RNA from light grown plants. Both EcoRI DNA fragments were cloned into the EcoRI site of pACYC184. Using these recombinant DNA, photogene I (EcoRI DNA fragment 4.8 Kbp) has been mapped in the SalI DNA fragment A and is located at approximately 8 Kbp from the 5' end of 23 rRNA gene. The photogene I contains internal sites for BamHI, PstI, HindIII, XhoI, and AvaI. Photogene II (EcoRI DNA 5.5 Kbp fragment) has been mapped on SalI DNA fragment B and is located at approximately 5 Kbp from the 3' end of the large subunit Ribulose bisphosphate carboxylase gene. The photogene II contains internal sites for PstI and ClaI. Photogene I and II have been found to transcribe RNA of 1 Kb and 1.7 Kb, respectively. Molecular sizes of the RNA transcripts of these genes has been confirmed by R-loop analysis.

28
GENETIC AND METABOLIC BASES FOR HERBICIDE (PARAQUAT) RESISTANCE
Hughes, Karen W.*, Dept. of Botany, University of Tennessee, Knoxville, TN 37996-1100

Cell lines of Nicotiana tabacum and Glycine max which are resistant to normally lethal levels of the herbicide Paraquat (1,1-dimethyl-4,4' dipyridylium salt) were selected from cell cultures. Some of the tobacco cell lines were regenerated into plants. Plants were either herbicide resistant or herbicide sensitive but in all cases, callus derived from R1 plants was resistant. Metabolic bases for resistance in paraquat-resistant cell lines included; 1. Reduced incorporation of the herbicide. 2. Increased levels of either superoxide dismutase, peroxidase, or both. Herbicide-resistant plants have been self pollinated and crossed with non resistant plants. Seeds from crosses of three different paraquat-resistant plants indicate that different genetic bases for the trait exist. In one case, the trait segregates as a single dominant gene. Seed segregation ratios for the other two mutants indicate polygenic inheritance involving 2 loci.

29
CHLOROPLAST DNA REARRANGEMENTS ARE MORE FREQUENT WHEN A LARGE INVERTED REPEAT SEQUENCE IS LOST
Palmer, Jeffrey D., & William F. Thompson*, Carnegie Inst. of Washington, Department of Plant Biology, Stanford, CA 94305.

We have examined the arrangement of sequences common to seven angiosperm chloroplast genomes. The chloroplast DNAs of spinach, petunia and cucumber are essentially colinear. They share with the corn chloroplast genome a large inversion of approximately 50 kb relative to the genomes of three legumes, mung gean, pea and broad bean. There is one additional rearrangement, a second, smaller inversion within the 50 kb inversion, which is specific to the corn genome. These two changes are the only detectable rearrangements which have occurred during the evolution of the five species examined (corn, spinach, petunia, cucumber and mung bean) whose chloroplast genomes contain a large inverted repeat sequence of 22-25 kb. In contrast we find extensive sequence rearrangements in comparing the pea and broad bean genomes, both of which have deleted one entire segment of the inverted repeat, and also in comparing each of these to the mung bean genome. Thus, there is a relatively stable arrangement of sequence in those genomes which feature the inverted repeat and a much more dynamic arrangement in those that have lost the inverted repeat.

30
IN VITRO MULTIPLICATION OF SOYBEAN (GLYCINE MAX L. MERRILL)
Kahlon*, Prem S., Sarabjit M. Bhatti and Therese M. Curry, Biology Dept., Tennessee State Univ., Nashville, TN 37203 USA

Soybean seeds of cultivars Centennial, Miles, Tracy and Williams were surface sterilized with 50% Clorox for 8 minutes and inoculated on Murashige-Skoog's (MS) medium (1962), containing cytokinin (6-benzylaminopurine-BA) and/or auxin (Indole-3-butyric acid-IBA). Intercotyledonary nodes from the conditioned seedlings were inoculated on MS media containing various combinations of 6-benzylaminopurine, naphthaleneactic acid and coconut water. Highest frequency of shoot-buds was obtained with low concentrations of BA (0.1 uM and 1.0 uM). The shoot buds or shoots thus obtained were subcultured on various media as follows: a) multiple buds were inoculated b) multiple buds were separated and inoculated individually and c) terminal and subterminal nodes were excised and inoculated individually. All of these inocula produced multiple shoot buds, however, the highest frequency of buds was obtained when terminal or subterminal nodes were excised and subcultured. Plantlets obtained from these studies could be potted or used for other studies, e.g. this micropropagation technique is a useful tool for In Vitro stress tolerance screening.

31

IN VITRO TRANSLATION OF THE MAJOR TUBER GLYCOPROTEIN OF POTATO

Park, William*, David Hannapel, Greg Mignery, and Edilson Paiva
Department of Horticulture, Purdue University, West Lafayette, Indiana 47907.

The major protein component of potato tubers is a family of glycoproteins with apparent molecular weights of approximately 40,000 daltons. This group of proteins has been given the name patatin. Patatin makes up 40-45% of the soluble protein in mature tubers, but is present in trace amounts, if at all, in other tissues.

We have isolated polyribosomes and poly(A) containing mRNA from stems and developing tubers and have translated them in vitro. By immunoprecipitation we have found that patatin is synthesized on membrane bound polysomes apparently as a precursor with slightly greater molecular weight. Patatin accounts for 30-40% of the in vitro translation products of RNA isolated from membrane bound polysomes from developing tubers, but is not present in detectable amounts in the translation products of either membrane bound or free polysomes from stems.

32

EFFICIENT SOMATIC EMBRYOGENESIS AND PLANT REGENERATION FROM OVULAR CALLUS OF CARICA PAPAYA L.

Litz*, Richard E. & Robert A. Conover, University of Florida, Agricultural Research and Education Center, 18905 SW 280 St., Homestead, FL 33032 USA.

Embryogenic callus has been isolated from Carica papaya L. ovules following controlled pollination between C. papaya and C. cauliflora Jacq. Ovules 20-140 days after pollination have been cultured on MS medium with 60 g/l sucrose, 400 mg/l glutamine and 20% (v/v) coconut water. Evidence for induction of polyembryony usually occurs 30-40 days later. Highly efficient somatic embryogenesis occurs upon transfer of ovular callus to MS medium with 60 g/l sucrose and 400 mg/l glutamine alone. Upon transfer of mature embryos to solid MS medium with 0.01-0.20 mg/l BA and 0.5-2.0 mg/l NAA, concurrent root and shoot formation occurs. We have also selected an embryogenic callus line that is responsive to 2,4-D (2 mg/l). Somatic embryos from this medium can be induced to germinate in liquid MS medium with 60 g/l sucrose and 400 mg/l glutamine.

33

THE ALCOHOL DEHYDROGENASE SYSTEM IN MAIZE

Pryor, A., E. Dennis, W. Gerlach, H. Lorz, M. Sachs, & J. Peacock, Division of Plant Industry, CSIRO, Canberra, AUSTRALIA

The two unlinked alcohol dehydrogenase genes (Adh1 and Adh2) in maize provide a powerful system for the study of gene organization and expression, for the development of plant gene transformation and for the characterization of mobile genetic elements in plants.

cDNA clones for both the ADH1 and ADH2 proteins have been isolated. Base sequence analysis shows that amino acid coding regions share 80% homology, but there is no homology between 3' untranslated regions. Certain Adh1 alleles have two mRNA size classes, while other alleles have only the smaller mRNA species. Southern digests indicate there to be only one or a few copies of each gene in the genome. Different ADH genotypes have different restriction patterns.

Callus cells derived from scutella tissue do not survive under anaerobiosis. Under the same conditions ADH+ cells survive and can be regenerated into plants. This observation provides a basis for the selection of transformed cells (ADH+) and may enable the introduction of other genes with the Adh+ gene.

34

INTERACTION OF AGROBACTERIUM WITH TISSUE CULTURE CELLS

Matthysse, Ann G.* & Robin H.G. Gurlitz, Botany Department, University of North Carolina, Chapel Hill, NC 27514 USA

The first step in tumor formation by Agrobacterium tumefaciens is the site-specific attachment of the bacteria to the plant host. We have demonstrated that virulent strains of A. tumefaciens are capable of specific attachment to plant tissue culture cells. The bacteria attached to cells of dicots (carrot, tobacco, and Vinca) in which the bacterium is virulent. A. tumefaciens neither attached in culture to, nor induced tumors on monocots (oat and corn) or carrot embryos. Thus one factor limiting the host range of the bacteria may be the presence of receptors on the plant cells. Receptors on the surface of carrot cells were sensitive to digestion by trypsin and chymotrypsin and could be extracted with dilute detergent. The carrot cells recovered the ability to bind bacteria 3 hours after enzyme treatment. By examining the attachment of a non-aggregate forming cellulose-minus mutant of A. tumefaciens the number of receptor sites on a carrot cell was estimated to be about 200.

35

EVALUATION OF CHLOROPLAST DNA HOMOLOGY TO NUCLEAR DNA IN NICOTIANA TABACUM

Schaefer, V. G.* and J. P. Miksche, Botany Dept., NCSU, Raleigh, NC 27650 USA

Chloroplast and nuclear DNA homology has been investigated utilizing ctDNA fragment inserts in the Pst I site of pBR322. Southern blot hybridizations were conducted between ^{32}P labeled nuclear DNA (nDNA) and cloned ctDNA fragments, as well as, between ^{32}P labeled ctDNA clones and Pst I nDNA restrictions.

36

WIDE SPECTRUM OF TISSUE CULTURE POSSIBILITIES WITH FERNS

Palta,H.K.,The New York Botanical Garden,Bronx,N.Y.10458

Ferns with independent gametophytic (sexual) and sporophytic (asexual) generations offer many exciting tissue culture possibilities.Rapid asexual propagation (cloning) is the major commercial application of this technology that interests fern growers,especially in case of Boston fern.Observations on rapid in vitro cloning of staghorn ferns will be presented,wherein agar-gelled, chemically-defined medium has been used.By repeated recycling the excised organs from plants produced from the parts of one initial plant,theoretical estimates project making available cloned material in excess of four million plants in one year.In vitro induction of apogamy and apospory is easy to achieve,and this can provide the route for producing new cytomorphotypes, some of which may have features that could be commercially useful.Breeders and growers would benefit by looking in to these possibilities because new forms and varities may be created by thesemeans.Information on tissue culture-induced autotetraploid sporophytes in Platycerium bifurcatum will be provided.Other aspects of fern tissue culture work emphasize that these plants provide an excellent material for basic research on problems of cellular differentiation, morphogenesis and genome expression.Because of high regeneration capability of both fern gametophytic and sporophytic tissues, somatic hybridization through interspecific fusion of protoplasts from either generation could yield novel plants.

37

TRANSPOSON Tn5-MUTAGENESIS IN RHIZOBIUM MELILOTI

G. Selvaraj* and V.N. Iyer, Dept. of Biology, Carleton Univ. Ottawa, Canada

Transposon mutagenesis is one of the effective methods in the genetic analysis of microorganisms which are less amenable to conventional methods. Van Vliet et al.(1978) and Beringer et al.(1978) have developed conjugative suicide plasmids which can function as carriers for delivering Tn5 into members of Rhizobiaceae. Our experience with one of these plasmids, pJB4J1 (Beringer et al.1978) indicated that the presence of bacteriophage Mu DNA on this plasmid contributed to lesions attributable to Mu. Meade et al.(1982) have also provided genetic and physical evidene to support this observation. To avoid the complications arising from the presence of Mu DNA on pJB4J1, we have constructed a suicidal plasmid independent of Mu. This plasmid pCU1O1::Tn5 is a derivative of pCU1O1. pCU1O1 is a recombinant plasmid carrying the entire tra genes of an IncN group plasmid pCU1 and the replicon of pACYC184 (Thatte & Iyer, in prep.). The N-transfer system of this plasmid is operative in E. coli x R. meliloti crosses, but the pACYC184-replicon being unstable in R. meliloti, a selection for neomycin-resistance specified by the transposon Tn5 yields transconjugants that carry Tn5-insertions. Using this suicidal plasmid, we have been able to derive auxotrophs of R. meliloti at a frequency of 4%.

38

PATHWAYS OF IN VITRO DIFFERENTIATION IN PEANUTS (ARACHIS SPP.)

Pittman, Roy N.*, Becky B. Johnson, and Donald J. Banks, CSRS, Langston University, Langston, OK 73050; Botany Department, Oklahoma State University, Stillwater, OK 74078; and USDA, ARS, and Agronomy Department, OSU, USA.

While several pathways of in vitro development have been reported for peanut (Mroginski et al., 1981), direct adventitious organogenesis or embryogenesis from callus has not been documented. We have obtained shoots from callus using agar medium with the minerals of Murashige and Skoog (MS) (1962), B-5 vitamins (Gamborg et al., 1968), and 1 mg/l each benzyladenine (BA) and naphthaleneacetic acid (NAA). Both cultivated and wild species have shown this response, with A. villosulicarpa producing the largest number of shoots.

Embryogenesis has occurred at a low frequency under several culture conditions. Callus derived from both immature leaflets and mature embryo axes have produced embryoids. Leaflets were cultured on the medium described above. Mature embryo axes were cultured on either MS medium with 7 mg/l kinetin and 18 mg/l indole acetic acid, or the medium of Saunders and Bingham (1972) with 2 mg/l each of kinetin, NAA, and 2,4-D.

39

AN INTEGRATIVE PLASMID OF PSUEDOMONAS SYRINGAE PV. PHASEOLICOLA

Szabo,* Les J. & Dallice Mills, Dept. of Botany and Plant Pathology, Oregon State Univ., Corvallis, OR 97331 USA

Halo blight of common bean Phaseolus vulgaris (L.) is incited by Psuedomonas syringae pv. phaseolicola. One strain of this pathogen has been shown to contain a 147 kb plasmid, pMC7105, which can replicate autonomously or integrate into the bacterial chromosome. Approximately 10 percent of randomly screened colonies derived from the integrated form contain an excision plasmid. Fifteen excision plasmids ranging from 33 kb to 250 kb have been characterized by restriction endonuclease and Southern hybridization analysis. These plasmids can be grouped into 5 classes on the basis of the sites at which excisive recombination occurs. Two of these classes are composed of F'-like plasmids which contain a portion of pMC7105 and chromosomal DNA from either side of the integration site. A third class contains all of pMC7105 and chromosomal DNA from both sides of the integration site. The integration site on pMC7105 has been identified on a 7.5 kb Bam HI fragment and cloned. This fragment has homology with other regions of pMC7105 and the plasmid-chromosomal juncture fragments. A 23 kb region from pMC7105 is common to all excision plasmids and presumed to contain the origin of replication. Auxotrophy and altered colony morphology have resulted from excision in two strains. The integrative property of this plasmid is being exploited to enhance our understanding of the genetics of this pathogen and to identify genes for virulence.

40

SENSITIVITY OF TOBACCO AND CARROT CULTURES TO INHIBTION BY AMA-TOXINS.

Preston,* James F. & Little, Michael C., Dept. Microbiology and Cell Science, University of Florida, Gainesville, FL 32611 USA

To obtain inhibitor resistant lines of Nicotiana species for selecting gene recombinants derived from somatic cell hybrids, suspension cultures of N. tabacum were evaluated for their sensitivity to inhibition by α-amanitin (AMA), cycloheximide (CHL), and parafluorophenylalanine (PFP). Using TdR incorporation as a measure of growth, AMA, CHL, and PFP were 50% inhibitory at 6.4×10^{-4}, 9.1×10^{-6}, and 10^{-3}M, respectively. Protoplasts derived from these cultures were inhibited 50% by 10^{-4}M CHL but were not affected by 9.1×10^{-3}M AMA. The conversion of AMA to 6'-O-methyl-α-amanitin (MeAMA) provided a derivative which was 10-fold more inhibitory to N. tabacum suspension cultures than the free AMA, and which was also an effective inhibitor of protoplasts. Suspension cultures of Daucus carota were inhibited 50% by AMA and MeAMA at 2×10^{-4} and 3×10^{-5}M, respectively. The greater inhibitory activity with MeAMA compared to AMA for both N. tabacum and D. carota suggests that α-amanitin may be subject to oxidase-mediated attack on the 6'-hydroxytryptophan moiety. Catechol oxidase levels were found to be 10-fold greater in N. tabacum compared to D. carota suspension cultures. The partially purified RNA polymerase II activity of N. tabacum demonstrated a K_I of 1.4×10^{-8}M with α-amanitin, further supporting the possibility of oxidase inactivation of α-amanitin by these cultures.

41

CHARACTERIZATION OF THE *CHL* A LOCUS OF *ESCHERICHIA COLI* K12: PROSPECTS FOR ITS USE IN A DNA TRANSFORMATION SYSTEM FOR PLANT CELLS

Taylor, Janet L.*, Andris Kleinhofs, John R. Bedbrook† and Frank J. Grant, Program in Genetics and Cell Biology, Washington State University, Pullman, Wash. 99164, USA, †Division of Plant Industry, C.S.I.R.O., Canberra, A.C.T. 2601, Australia

The presence of molybdenum cofactor activity in extracts of *E. coli* wild type and all chlorate-resistant mutants examined, except the *chl* A mutant, was demonstrated by *in vitro* complementation of the nitrate reductase (NR) deficient mutants *cnx*68 of *Nicotiana tabacum* and MA-2 of *Hyocyamus muticus*. The results of these *in vitro* complementation assays suggested that the *E. coli chl* A gene was a source of donor DNA for a plant cell DNA transformation system. The *chl* A gene was cloned on a 1.7 kb Bcl I fragment inserted into pBR322. This plasmid, pFG2, was restriction enzyme mapped and the *chl* A gene product identified using the maxicell protein synthesizing system. Extracts of the *chl* A mutant, transformed with the plasmid borne wild-type *chl* A gene, were able to restore NADH-NR activity to extracts of *cnx*68 and MA-2. These results indicated that the *chl* A gene codes for a protein product which corrected the mutation in *cnx*68 and MA-2 *in vitro*. (Supported in part by USDA CRGO Grant #79-00536 and NSF Grant PCM-8011348.)

42

Effects of Salt and Auxin Source on Ward Wheat Cell Suspension

Sharma, Govind C.,* Wen-Chung Wang and Caula A. Beyl

Murashige and Skoog (MS), Nitsch's (N), Gamborg's B-5 (B5), Heller's (H) and White's (W) media were evaluated for the ability to support growth of filtered cells containing nurse clumps of Ward wheat. The cell suspensions were subcultured every 7 days over a period of 5 weeks. The greatest increase in cell number (CN) and in clump fresh weight was observed using MS and N salts. The higher salt media with a larger proportion of ammonium favored both increase in CN and final fresh weight of clumps.

Picloram (0.25, 0.5, 1.0 mg/l) was compared with 2,4-D (0.75, 1.5, 3.0 mg/l) to determine its effect on growth of filtered cells containing nurse clumps. Use of picloram at the lower levels resulted in a lesser rate of CN increase but at 1.0 mg/l, it was equivalent in performance to 1.5 mg/l 2,4-D. An increase in packed cell volume (PCV) was observed with an increase in Picloram concentration. Highest PCV was found with 1.0 mg/l Picloram followed by 1.5 mg/l 2,4-D. The 3.0 mg/l 2,4-D concentration was repressive. Final clump dry weight showed that, in general, Picloram promoted clump dry weight increases particularly at the 0.5 mg/l level. Each of the 4 growth parameters measured, CN, PCV, fresh and dry weights of clumps, was affected differently by each hormone illustrating that there is no ubiquitous 'best' hormone concentration for all aspects of cell growth dynamics in suspension culture. Suspension established with filtered cells and no clumps declined rapidly irrespective of salt and auxin formulation of the media.

43

CLONING OF Bacillus thuringiensis GENES CODING FOR PESTICIDAL CRYSTALS

Wilcox, David R.*, Patrick Russo and Helen Eberle, Dept. RB&B, Univ. of Rochester, Rochester, NY 14642

Three strains of Bacillus thuringiensis (HD-1, HD-263 and HD-567) have been submitted to a cloning regimen that selects for genes coding for the B. thuringiensis pesticidal crystals (Schnepf and Whiteley, 1981 PNAS 78:2893-7). HD-1 and HD-263 are B. thuringiensis var kurstaki. HD-567 represents B. thuringiensis var israelensis which is effective against various Dipteran species. Potential clones have been isolated from each of the three strains. In Southern blot hybridizations the HD-1 clones show hybridization of DNA to the large plasmid regions of the gel. Analysis of several HD-1 clones has produced a detailed restriction map of the cloned region.

44

REGENERATION OF PLANTLETS BY SOMATIC EMBRYOGENESIS IN SANDALWOOD (Santalum album-L)

Dr.(Mrs.)Lakshmi Sita, Microbiology & Cell Biology Laboratory, Indian Institute of Science, Bangalore12

Somatic embryogenesis was induced from callus cultures of sandal shoots from mature trees. These embryoids have developed into plantlets with a well developed tap root system. Plantlets have established well in pots as well as soil and have grown to a height of 10 feet. Experimental conditions have been standardised during the last three years to have a switch-on switch-off system so that one could induce platlets at will. Cultures can be maintained at four stages: i. The callus phase where callus is maintained on MS medium supplemented with 1 mg/1,2, 4,D.; ii. Differentiation phase where embryogenesis can be induced within 15 days by subculturing callus from stage (i) to MS medium supplemented with 1 mg/ 1 GA; iii. Wherein embryoids are subcultured to whites medium supplemented with 0.5 mg/1 IAA, where a well developed platlet is formed; iv. Transfer of platlets to liquid medium with filter paper bridges, prior to potting.

Histological studies also were carried out to conclusively prove the bipolar nature of the embryoids. The results presented clearly demonstrate that the technique can used for the clonal propagation.

45

CLONING AND CHARACTERIZATION OF 2 LIGHT REGULATED GENES IN LEMNA GIBBA

Wimpee, Charles F.* and Elaine M. Tobin, Biology Dept., UCLA, Los Angeles, CA 90024 USA

We have constructed a genome library from the nuclear DNA of the aquatic plant, Lemna gibba. The library was screened using 2 separate ^{32}P-cDNA preparations synthesized from size-selected poly(A)RNA. This RNA was enriched for the mRNAs for 2 light-regulated chloroplast proteins: the chlorophyll a/b protein and the small subunit (SSU) of RuBP carboxylase. We have isolated several clones containing what appear to be 3 separate a/b genes and a number of overlapping clones containing one SSU gene. The identity of these clones was verified by hybridization to cDNA clones containing the a/b and SSU sequences, hybridization to RNA gel blots, and translation of hybrid-selected RNA followed by immunoprecipitation. We have demonstrated that ^{32}P-labelled a/b clones hybridize to a much greater extent to RNA isolated from plants which have been grown under constant light than to RNA isolated from plants which have been placed in the dark for 7 days. Preliminary characterization of these clones will be presented.

46

CRYOPRESERVATION OF CULTURED PLANT GENOTYPES AT -196°C

Ulrich, Jane M.* and Bernard J. Finkle, Western Regional Research Center, USDA-ARS, Berkeley, CA 94710 USA

Valuable lines of plants, including mutants and hybrids, can be preserved during long periods of time in liquid nitrogen with little or no change in genetic state and viability. Freezing germplasm minimizes genetic drift, saves space and the time and material required for media preparation, and avoids contamination and the need for frequent transfers.

Gradual addition of 10% polyethylene glycol (Carbowax 6000), 8% glucose and 10% dimethylsulfoxide (DMSO) to actively growing cell cultures, followed by a programmed lowering of temperature to -30°C then transfer to liquid nitrogen (-196°C) gave viable cells for many species when thawed rapidly at 40°C, and washed at room temperature (22°C). Documentation will be presented showing the benefits of using a mixture of cryoprotectants, and from using a 22°C wash solution in preference to a cold wash. Photographs will be shown of flowering alfalfa plants and young date palm trees which developed from callus thawed after freezing at -196°C.

47

COMPUTERIZED ANALYSIS OF TWO-DIMENSIONAL ELECTROPHORETOGRAMS OF EMBRYONIC SOYBEAN PROTEINS

Hruschka, William R.*, David R. Massie*, and James D. Anderson**, *Instrumentation Res. Lab, BARC, USDA, Beltsville, MD 20705 USA, **Plant Hormone Lab, BARC, USDA, Beltsville, MD 20705 USA

Soluble proteins from soybean embryonic axes were radioactively tagged and separated by the high-resolution gel electrophoresis method of O'Farrell. Six replicate electrophoretograms of the same protein mixture were produced. An inhouse-designed digitizer consisted of a photographic enlarger projecting the image onto the bed of a programmable plotter with a photocell detector attached to the penholder. A desktop computer controlled the pen and collected the intensity readings of an 8x8 cm portion of the gel image into a 256x256 array which was then transferred to a minicomputer for analysis. Fortran programs were developed which reproduce the original autoradiograph patterns and quantify individual proteins. After smoothing and removal of background, the integrated intensity of each protein had digitization error of less than 2% and the gel-to-gel variation was less than 15%. An on-line demonstration of the program will be provided.

48

A CELL-FEEDING TECHNIQUE FOR ENSURING SUCCESS OF PROTOPLAST CULTURE IN SUGARCANE

Chen, Wen Huei & Soh Chao Shih, Taiwan Sugar Research Institute, Tainan, Taiwan 700, ROC

The general culture conditions which are usually used for other hgiher plants' protoplasts would result in a rapid lysis of sugarcane protoplasts within 48 hr incubation. Therefore, a cell-feeding technique, use of suspension cells as feeder layer to support the division of an upper layer of cultured protoplasts was further developed and worked well. Cultured protoplasts on the feeder layer enlarged several times in volume or proceeded budding within 3 days cultivation. After 5 days incubation, approximately 10% of the protoplasts regenerated cell wall and underwent their first division. 10 days later, multiple division were commonly observed. Small colonies of cells appeared on the upper layer of the plates after an additional 10 days incubation. The use of the cell-feeding technique for protoplast cultures in cereal crops is discussed.

49

SEASONAL INFLUENCE ON SHOOT RNA IN DIFFICULT VS. EASY TO REGENERATE PLANTS Davies*, Fred T., Jr., Department of Horticultural Sciences, Texas A&M University, College Station, TX 77843 USA

Seasonal influence on maximizing regeneration potential is critical in the asexual propagation of many important woody plant species. The relationship of shoot RNA to seasonal differences in adventitious root formation was studied in juvenile and mature Ficus pumila. Azure B was used in the differential staining for RNA following the procedures of Jensen. RNA was quantified with a Zeiss scanning microspectrophotometer at 688-655 nm with 160x. Scans in the shoot apicies encompassed the tunica and part of the corpus region; 45-90 cells were scanned, depending on meristem size. Both easy-to-root juvenile and difficult-to-root mature F. pumila had higher shoot RNA levels during peak rooting periods. Highest RNA was recorded with juvenile material during peak rooting periods, while lowest RNA was found in mature shoots during low rooting intervals.

50

ABSTRACT: Protoplast Isolation of Loblolly pine & Shortleaf pine HUANG, F.H. & SONIA TSAI, U of A, Fayetteville, AR 72701 U.S.A.

In their shortleaf x loblolly hybrids study, Mr. Tim La Farge and John Krans have used a number of F_1 and backcrosses between these species. What they attempted to accomplish is to transfer "resistance of fusiform rust" from slower growing bud immune shortleaf pine to the fast growing but highly susceptible loblolly pine. In the Forestry Genetics laboratory at the University of Arkansas, we are attempting an unconventional method i.e., protoplast hybridization, to produce hybrids between loblolly pine and shortleaf pine in the hope that the hybrids will retain the merits of both species.

The protoplasts are isolated from cultured cells which are initiated by placing the splitted hypocotyls of young seedlings on Brown and Lawrence medium. Procedures of protoplast isolation are modified from "Driselase for protoplast preparation". During the experiment, we used 20-fold concentration of "Driselase" as digesting enzyme over the amount recommended in the "Driselase for protoplast preparation" for pine callus protoplast isolation. Viable, spherical protoplasts of pine are observed under the light microscope. The pine callus cells are quite elongated before removing the cell walls. Although isolation of protoplast from pine needles has not been undertaken, we believe the procedures for isolating protoplasts from cultured cells are applicable in the isolation of protoplasts from pine needles.

51

SOMATIC EMBRYOGENESIS AND PLANTLET DEVELOPMENT IN ANTHER CULTURE OF Cucumis Sativus L.
Lazarte*, Jaime E. and Carole C. Sasser, Horticultural Sciences, Department, Texas A&M University, College Station, Texas 77843, USA.

Callus developed after culturing anthers for 3-4 wks. on a modified Nitsch & Nitsch basal medium. Further calli proliferation and greening was obtain after 2-4 wks by subculturing on modified Murashige & Skoog medium with 1.0 mg/liter 6 benzylamino purine and 0.1 mg/liter gibberellic acid. Callus masses were separated and placed on Nitsch & Nitsch medium with 0, 1 mg/liter Kinetin. Dark green areas subsequently differentiated into embryoids. Plantlets development occured after embryoids were transferred to Nitsch & Nitsch medium without growth regulators.

52

THE ENDOSYMBIOTIC CYANELLE FROM CYANOPHORA PARADOXA: A MODEL FOR CHLOROPLAST EVOLUTION

Löffelhardt, W., Mucke, H., Bohnert, H.J., Institut für Allgemeine Biochemie, Wien, Austria, and EMBL, Heidelberg, F.R.G.

Cyanelles from C. paradoxa correspond to plant chloroplasts as far as their genome size and organization is concerned. Yet several morphological and biochemical criteria point to their origin as cyanobacterial invaders of a flagellate host, the most striking point for this view is a peptidoglycan layer as a cell wall remant.

Improved methods of cyanelle isolation and DNA-purification show that the size of cyDNA is equivalent to 124 kbp in one strain and 139 kbp in a second. A rather different DNA pattern after restriction endonuclease digestion of the two cyDNAs shows that the event of cyanobacterial invasion might have happened more than once, although sequence homologies between the two cyDNAs have been revealed. A physical map of cyDNA has been constructed showing a repeat unit which contains the rRNA genes. The positions of genes coding for proteins are included in this map.

53

LIPOSOME-MEDIATED INTRODUCTION OF DNA INTO PLANT PROTOPLASTS
Lurquin, Paul F., Program in Genetics and Cell Biology, Washington State University, Pullman, WA 99164 USA

Recombinant plasmid DNA of molecular weight up to 38 kb pairs has been encapsulated in neutral, positively and negatively charged multilamellar and unilamellar liposomes. Phosphatidylserine/cholesterol reverse evaporation vesicles (PS/CH REV) and phosphatidylcholine/stearylamine multilamellar vesicles (PC/SA MLV) had the highest sequestration capacities. Both types of liposomes were shown to strongly interact with cowpea and tobacco mesophyll protoplasts and with tobacco and carrot protoplasts isolated from suspension cultures. Binding of liposomes and transfer of sequestered DNA was shown by scanning electron microscopy, autoradiography, fluorescence microscopy, subcellular fractionation and marker rescue experiments. Liposome binding was enhanced by polyethylene glycol and was highest at pH 5.6 in the case of PC/SA MLV and at pH 9.0 with PS/CH REV. High ionic strength strongly inhibited the binding of PC/SA MLV but had little effect on PS/CH REV. Ca^{++} enhanced PS/CH REV binding. Analysis in metrizamide gradients showed complex formation between transferred plasmid DNA and nuclear proteins. DNA transferred into protoplasts through PS/CH REV fusion underwent less extensive degradation than PC/SA MLV transferred DNA.

54

CELL FUSION FOR TRANSFER OF CHLOROPLASTS, AND OF CYTOPLASMIC MALE STERILITY IN *NICOTIANA*
Pál Maliga*, Ferenc Nagy, Ágnes Csépl0, Péter Medgyesy, Gabriella Lázár & László Menczel
Institute of Plant Physiology, BRC, Szeged, P.O.Box 521, Hungary, H-6701

Plastids were transferred from irradiated *N.tabacum* into *N.plumbaginifolia* cells by rescue of the plastome coded SR1 streptomycin resistance mutation (1). By selection for streptomycin resistance cytoplasmic male sterility, a mitochondrial trait, could also be transferred (unpubl.). Directed transfer of plastids was achieved without selection irradiating the donor, and treating the recipient with iodoacetate (2). Plastid transfer was also obtained by fusing SR1 cytoplasts (enucleated protoplasts) with sensitive *N.plumbaginifolia* cells (3).

Lincomycin resistance isolated in *N.sylvestris* was rescued into *N.plumbaginifolia* plants and, based on co-segregation, shown to be a plastome trait.

Protoplast fusion was followed by extensive rearrangements in the mitochondrial DNA of some (4) but not all (*N.tabacum+N.sylvestris*) somatic hybrids.

1. Menczel et al.Genetics,in press; 2. Sidorov et al. (1981) Planta 152,341; 3. Maliga et al. MGG, in press 4. Nagy et al.(1981) MGG,183,437

55

TISSUE CULTURE STUDIES IN BROMEGRASS (BROMUS INERMIS)

Molnar, Stephen J.* and John L. Grainger, Genetic Engineering Section, Ottawa Research Station, Agriculture Canada, Ottawa, Ontario, Canada, K1A 0C6

In this communication we report the results of our studies to develop and optimize techniques for the initiation and maintenance of long term totipotent cultures of bromegrass cv. Manchar. Organized, complex cultures were initiated efficiently from surface-sterilized seeds placed directly onto the culture media of Shenk and Hildebrandt (1972) or Murashige and Skoog (1962) supplemented with 0.5 to 7.0 mg/L 2,4-D. Kinetin added at one-tenth the auxin concentration had little effect. Numerous embryos arose spontaneously on approximately 25% of the initial cultures. Similar cultures were initiated from other explants including the apical meristems of seedlings and immature inflorescences and with other auxins, including p-chlorophenoxyacetic acid. Plantlets were regenerated from such embryos and from early passage embryo-derived suspension cultured cells placed on growth regulator-free culture media.

56

USE OF PICLORAM AS AUXIN IN ONION (ALLIUM CEPA) TISSUE CULTURES

Phillips, Gregory C., Dept. of Horticulture, New Mexico State Univ., Las Cruces, NM 88003-3530 USA

4-Amino-3,5,6-trichloropicolinic acid (picloram) was compared to 1-naphthaleneacetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D) as auxin sources for establishing in vitro shoot propagation and callus cultures, respectively, from cultivated onion (Allium cepa L.) seedlings, cvs. 'Yellow Grano' and 'Yellow Sweet Spanish.' Differences in response to the various auxins were noted among genotypes of both cultivars. N6-Benzyladenine (BA) was used as cytokinin in all experiments at concentrations of 2.2 uM to 8.8 uM. Considering all genotypes tested, picloram (0.1 to 0.25 uM) was superior to NAA (0.3 to 3.0 uM) for rapid clonal propagation of shoots. Picloram did not inhibit rooting of shoots compared to NAA. Picloram (0.25 to 3.75 uM) was superior to 2,4-D (0.65 uM) for callus proliferation. Picloram did not inhibit subsequent morphogenesis from callus compared to 2,4-D. Somatic embryogenesis was observed in 7-month-old callus growing on medium containing 3.75 uM picloram and 2.2 uM BA. Little morphogenesis was observed in control callus growing on medium containing 2,4-D. Development of procedures for plant regeneration from long-term cultures should provide a basis for applying cellular selection procedures in onion improvement programs.

57

GENETIC ANALYSIS OF SYMBIOTIC NITROGEN FIXATION GENES

F. Ausubel*, W. Buikema, C. Earl, S. Long & G. Ruvkun, Department of Cellular and Developmental Biology, Harvard University, Cambridge, MA 02138 USA

A 100 kilobase region of the R. meliloti genome adjacent to and surrounding the genes for nitrogenase (nif genes) was cloned and the recombinant plasmids were used to construct Tn5 mutations in the R. meliloti nif region. Each of the Tn5 insertions was assayed for its effect on the ability of R. meliloti to nodulate alfalfa (Nod phenotype) and to symbiotically fix nitrogen (Fix phenotype). Tn5 insertions immediately adjacent to the nitrogenase genes which caused a Fix phenotype (Fix ::Tn5) were found in two clusters, one of 6.3 kb and a second of at least 5.0 kb. We found that the R. meliloti genes nifH, nifD, and nifK, which code for the single subunit of the nitrogenase Fe protein and for the two subunits of the nitrogenase MoFe protein respectively, are located in the 6.3 kb Fix ::Tn5 cluster. These genes are transcribed in the order nifH, nifD, nifK, which is the same order as in K. pneumoniae. Tn5 insertions which caused a Nod phenotype were found in a cluster only 20 kb away from the nitrogenase genes. The nod and fix genes identified in this study are located on a large indigenous plasmid which is probably at least 500 kilobases.

58

DIHAPLOIDS VIA ANTHERS CULTURED IN VITRO

P. S. Baenziger*and G. W. Schaeffer, Field Crops and Cell Culture and Nitrogen Fixation Laboratories, BARC-W, Beltsville, MD, 20705 USA

General methods have been developed for the recovery of dihaploids. Anther culture is the most recent method and can be used to recover large numbers of haploid plants in several crops. Techniques for developing dihaploids via anther culture, the breeding theory behind their use, how dihaploids are being used in breeding programs, and their future uses were discussed. The main breeding advantages of dihaploidy is rapid derivation of homozygous lines and selection among those lines in the absence of dominance variation. The main disadvantage is large evaluation nurseries are required. Most of the dihaploids from anther culture used in breeding programs have been derived from tobacco, though good progress has been made in wheat and rice. In tobacco, the technique may induce variation which is usually deleterious. Dihaploids derived from interspecific hybridization (barley and preliminarily tobacco) do not exhibit as much deleterious variation as observed with anther culture indicating the variation may be caused by the technique for developing dihaploids and not dihaploidy itself. The expectation is that useful variation may also be recovered. The potential for dihaploids in biochemical selection, mutation breeding, and gene transfer systems were also discussed.

59

EXPRESSION OF α-AMYLASE GENE IN BARLEY ALEURONE CELLS.

Chandra, G.R., Seed Research Lab., USDA-ARS, Beltsville, MD 20705; Muthrishnan, S., Dept., of Biochemistry, Kansas State Univ., Manhattan, KS 66506, USA.

In barley aleurone layers, the addition of gibberellic acid causes an increase in the level of translatable α-amylase mRNA. The α-amylase mRNA is determined to have 1500 nucleotides. cDNA was cloned at the Pst I site of plasmid pBR322 by the G-C tailing technique. The probe DNA has 630 base pairs. A "Southern blot" of barley embryo DNA digested with different restriction enzymes when probed with 'nick-translated' α-amylase clone DNA revealed the presence of multiple genes for α-amylase in barley. The invivo formation of α-amylase mRNA was dependent either on the exogenous addition of gibberellic acid or the endogenous supply of gibberellic acid from the scutellum.

60

HYBRID AND CYBRID PRODUCTION VIA PROTOPLAST FUSION.

Cocking, Edward C., Plant Genetic Manipulation Group, Department of Botany, University of Nottingham, Nottingham, NG7 2RD, U.K.

Protoplasts can be fused using a range of chemical fusogens, and also by electrical procedures; and the resultant heterokaryons can sometimes be suitably cultured to form hybrid plants. To determine factors influencing the degree of nuclear hybridity we have developed a simple procedure for the mechanical isolation of heterokaryons using both bright field and fluorescence procedures; (Plant Science Letters: 24, 105-110 (1982)); we are also utilising a nitrate reductase deficient mutant, coupled with fusion with irradiated protoplasts, to regulate the degree of nuclear hybridity.

Cybrid production by protoplast fusion is also being assessed utilising both irradiation procedures and enucleate subcellular units, such as microplasts (Protoplasma: 110, 147-151, (1982)). Somatic hybrid and cybrid plants produced by these unconventional genetic manipulation procedures will be of importance in plant improvement programmes.

The range of agricultural applications from protoplast fusions is extensive (Phil. Trans. R. Soc. B. 292, 557-568 (1981)), and this aspect of plant genetic manipulations can now be developed, provided there is adequate cultural capability.

61

Fedoroff, N.V., Carnegie Institution of Washington, Department of Embryology, 115 West University Pky., Baltimore, MD 21210

Molecular studies have been carried out on mutations caused by the transposable element Ds at the Shrunken (Sh) locus in maize. Three mutant alleles have been examined and all have been found to have structural alterations in the immediate vicinity of the transcription unit. The structural alterations map near the 5' end of the transcription unit in two of the mutants. Both of the mutants produce 0.5-1% of the normal level of the mRNA encoded by the Sh locus and produce undetectable quantities of sucrose synthetase, the enzyme encoded by the locus. The third mutant contains a rearranged transcription unit in which the 5' and 3' coding sequences are separated by an insertion or rearrangement. Aberrant transcripts of the locus are present in endosperm tissue of this mutant. The transcripts are slightly shorter than the Sh-encoded sucrose synthetase mRNA, are missing the coding sequence beyond the rearrangement breakpoint, and encode two polypeptides which are antigenically related to sucrose synthetase, but have a slightly higher mobility on denaturing gels.

62

ORGANIZATION AND EXPRESSION OF NUCLEAR GENES.

Richard B. Flavell, Plant Breeding Institute, Trumpington, Cambridge CB2 2LQ , England.

The purification of DNA sequences from higher plant chromosomes using recombinant DNA techniques has already revealed a wealth of new information about the structure and organization of the many different kinds of nucleotide sequences that make up the large, complex chromosomes. Some of the principal findings will be reviewed together with their implications for understanding the molecular basis of genetic variation on which plant breeding programs are based. Recent studies on genetic variation affecting the structure, number and activity of ribosomal RNA genes in wheat will be included as examples. New opportunities in plant genetics and plant breeding arising from the genetic engineering of chromosomal sequences will be discussed.

63

UTILIZATION OF GENETICALLY-ENGINEERED MICROORGANISMS FOR THE MANUFACUTRE OF AGRICULTURE PRODUCTS

Glick, J. Leslie & Peirce, M. Virginia, Genex Corporation, 6110 Executive Boulevard, Rockville, MD. 20852

Genetically engineered microorganisms will soon play a significant role in the manufacture of products used in agriculture as well as products obtained from agriculture. Many products currently used in producing crops and in raising livestock, such as pesticides, herbicides, vitamins and antibiotics, can potentially be produced more economically, and in higher yields, by appropriately developed microbial strains. Furthermore, agricultural products not widely used due to prohibitive manufacturing costs will become more available, profoundly affecting agricultural practices. For example, microbially produced animal growth hormones and vaccines to prevent diseases affecting livestock are already being tested. Additionally, it is anticipated that microbial production of expensive amino acids, such as tryptophan and threonine, could reduce their respective per pound cost ten to twenty fold, thereby stimulating demand for them as feed supplements. Products obtained from agriculture, such as high value added plant chemicals used in the manufacture of drugs, may also be produced more economically by genetically engineered microorganisms. The feasibility of the development of these new processes depends on technical and economic criteria and will require interdisciplinary cooperation.

64

A BIOSYNTHETIC POLYPEPTIDE VACCINE FOR FOOT-AND-MOUTH DISEASE.

Douglas M. Moore, USDA-ARS, Plum Island Animal Disease Center, Greenport, NY 11944

Worldwide demand for foot-and-mouth disease (FMD) vaccine remains high. One approach to satisfy this demand is the biosynthesis of the immunogenic outer capsid polypeptide, VP_3, for use in vaccine. Complementary transcripts of the FMDV A_{12} genome were cloned into E. coli, the coding sequence of VP_3 was identified and a plasmid constructed to express VP_3 as an E. coli-VP_3 chimeric protein. The protein was produced in high yield and when used in a vaccine, protected cattle and swine against FMD virus challenge. Cloning of additional strains provided their respective VP_3 amino acid sequences and distinct serotype and subtype variable zones were found. This together with antigenic analysis of VP_3 fragments and capsid surface site studies, locates the position of exposed capsid antigenic sites within the primary structure of VP_3.

65

REGENERATION OF TRANSFORMED CELLS

Owens, Lowell, Dean Cress, and Esra Galun, USDA-ARS, Cell Culture & Nitrogen Fixation Lab, Beltsville, MD, 20705, USA, and Dept. of Plant Genetics, Weizmann Inst. of Science, Rehovot, Israel.

We have reported the infrequent occurrence of teratoma formation from cultured octopine-type crown gall of tobacco based on the criteria of octopine production and hormone-independent growth. Recently, DNA hybridization analysis has revealed that T-DNA is present in leaf tissue of these teratomas. The objective of the present study was to determine whether the teratomas, which originated from non-cloned crown-gall calli, actually arose from transformed tumor cells or were merely chimeras of tumor and normal or revertant cells. Sixty eight single-cell clones were obtained by culturing teratoma leaf protoplasts. Of these, 62 clones regenerated teratomas when cultured on hormone-free medium, and each produced octopine. In a separate experiment, 10 leaf explants of the original noncloned teratoma line were forced to grow as calli by incorporation of hormones into the medium. After 6 months, the 10 calli sublines were returned to hormone-free medium. All 10 calli proceeded to regenerate teratomas whose leaves produced octopine. From these two lines of evidence, we conclude that the intitial octopine-type teratoma originated from a variant transformed cell.

66

VIROID cDNA - USES IN VIROID DETECTION AND MOLECULAR BIOLOGY

Owens*, Robert A. and T. O. Diener, Plant Virology Laboratory, Beltsville Agricultural Research Center, Beltsville, MD 20705 USA

Viroids are a novel class of subviral pathogens-unencapsidated small circular RNAs that can be isolated from plants afflicted with specific diseases. As the emphasis in viroid research begins to shift from physical-chemical studies of viroid structure to studies of mechanisms of viroid-host interaction, cloned viroid cDNA has proven to be a very versatile tool.

Potato spindle tuber disease poses a potentially serious threat to efforts to adapt the potato to growth in subtropical and tropical climates. Exclusion of potato spindle tuber viroid (PSTV) from seed potatoes will be essential, because the presence of PSTV causes severe damage, even total crop loss, in warm climates. However, disease diagnosis has often been very difficult. Hybridization between highly radioactive recombinant PSTV cDNA and viroid RNA that has been attached to a solid support provides a sensitive and reliable new method for the detection of PSTV.

Current efforts to identify regions of the viroid RNA sequence that are involved in viroid-host interaction(s) depend upon comparisons of the sequences of known viroids. Directed in vitro mutagenesis of cloned viroid cDNAs offers a promising alternative approach.

67

MITOCHONDRIAL DNA PLASMIDS AND CYTOPLASMIC MALE STERILITY.
Pring, D. R., ARS-USDA, Dept. of Plant Pathology, Univ. of Fla., Gainesville, FL., 32611 USA.

Plasmid-like circular or linear mitochondrial DNAs have been found in fertile and cytoplasmic male-sterile maize and sorghum, ranging in size from ca. 1-8 kb. Linear molecules of 6.4 and 5.4 kb are associated with one source of male sterility in maize, and linear molecules of 5.7 and 5.3 kb are found in one source in sorghum. Hybridization experiments indicate that the maize and sorghum molecules share some sequence homology, suggesting common genetic information. Smaller circular molecules of discrete size are also associated with sources of male sterility in maize and sorghum. Hybridization experiments indicate that the maize molecules may have derived by excision from normal cytoplasm mitochondrial DNA. Analyses of spontaneous male-fertile plants from male-sterile parents indicates a concomitant integration of sequences of the plasmid-like DNAs into the principal genome. These analyses suggest a transposon-like capability of the DNAs. Studies with other sources of male sterility in maize show a gradation of homology to the plasmid-like DNAs, which may suggest a correlation with the biology and genetic behavior of male-sterile maize cytoplasms.

68

MUTATIONS AND CELL SELECTIONS
G. W. Schaeffer, Cell Culture and Nitrogen Fixation Laboratory, BARC-W, Beltsville, MD, 20705, USA.

The rapid progress in microbial genetics in past decades was due in part to short cell cycles and the availability of mutants which prescribed the biochemistry. Hypothetically large numbers of separated plant cells in liquid suspensions provide some of the same benefits to plant biologists previously enjoyed by microbiologists. However, in actual practice plant systems are much more complex, cells rarely exist as single cells and gene expression is complex and controlled by developmental processes. Plant biochemists urgently need mutants of biochemical events for systematic and predictable progress in plant modifications through somatic cell fusion, gene isolations and gene transfer. The report reviews briefly the current status of plant mutant selections in cell culture, and discusses the potentials and pitfalls of *in vitro* techniques. The work illustrates useful and deleterious variability recovered by the *in vitro* culture of rice cells.

69

GENE VECTORS FOR HIGHER PLANTS
Schell, J.*, Van Montagu, M., Hernalsteens, J.P., De Greve, H., Leemans, J., Willmitzer, L., Otten, L., Schröder, J. and G., Laboratorium voor Genetika, RU, Gent, Belgium - MPI für Züchtungsforschung, Köln 30, FRG

Ti-plasmids, carried by *Agrobacterium tumefaciens* have been shown to be responsible for Crown gall formation in Plants. Ti-plasmids are natural gene-vectors with which Agrobacteria achieve the transfer and stable maintenance of a defined DNA segment (called T-region) into the nucleus of transformed plant cells. Using site specific mutagenesis it was possible to introduce mutations in different parts of the T-region. The transcription of the T-DNA in wild-type and mutant Crown galls was compared and it was found that the induction of specific developmental patterns could be correlated with the absence of specific T-DNA transcripts. Double mutants were obtained in which the expression of all the "onc" genes was abolished. Tobacco, potato and Petunia plant cells harbouring such inactivated T-DNAs were shown to regenerate normal, fertile plants that transmit the T-DNA segment as a single Mendelian locus. Structural genes, coding for opine synthase enzymes, were shown to be fully active. Several T-DNA genes were sequenced and transcription promotor and termination signals were identified.

70

MORPHOGENESIS AND REGENERATION IN TISSUE CULTURE
Thorpe, Trevor A., Department of Biology, University of Calgary, Calgary, Alberta, Canada, T2N 1N4

The application of plant tissue culture technology to agriculture and forestry requires the capacity to regenerate plants reproducibly in vitro. For some purposes direct regeneration from explants is suitable, but cells that have been genetically modified must be regenerated from cell lines or tissues maintained in culture. Regeneration is regulated via selective gene activity. However, the mechanisms involved in this control are as yet unknown. Nevertheless, this activity is reflected by changes in the tissue during primordium formation or somatic embryogenesis. In this talk, emphasis will be placed on the physiological and metabolic aspects of shoot primordium formation in tobacco callus. In particular the role of carbohydrate and nitrogen utilization and metabolism will be discussed. Carbohydrate has been shown to have dual roles as an osmotic agent and as an energy source during shoot formation. Studies on nitrogen assimilation and aromatic amino acid metabolism indicate enhancement during shoot primordium formation. There is also a greater requirement for reducing power. These findings are in keeping with the hypothesis that a shift in metabolism precedes and is required for organized development in vitro.

71

ORGANIZATION AND EXPRESSION OF HOST GENES INVOLVED IN SYMBIOSIS WITH *RHIZOBIUM*.
Verma*, D.P.S., D. Bewley, S. Auger and F. Fuller, Department of Biology, McGill University, Montreal, Canada H3A 1B1

Development of symbiotic root nodules on legume plants leading to the fixation of atmospheric nitrogen, is a result of coordinated expression and modulation of several host genes. We have identified a group of plant genes that are specifically induced following infection of plant by *Rhizobium*. These nodule-specific genes transcribe into a moderately abundant mRNA population which follows a similar kinetics of induction as leghaemoglobin. In addition, there are some sequences in root whose concentrations increase to about 20 fold during nodule development. Part of this response can be mimiced by treatment of the plant by a growth regulator, indole acetic acid (IAA). Two-dimensional gel electrophoresis of proteins from *Rhizobium*-infected and hormone-treated seedlings, labelled with ^{3}H-leucine, showed an increase in the synthesis of several peptides as well as appearance of one new peptide. Hormone treatment, however, does not influence the induction of leghaemoglobin or any other nodule-specific sequences. Molecular cloning of cDNA from nodules shows the presence in this library of sequences that are decreased in concentration and may represent another set of genes that are repressed following infection of the plant by *Rhizobium*.